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patient, the injured knee and the unharmed knee showed no difference in varus angle. In 5 patients the measurement of telomere lengths showed that telomere length of lateral femoral condyle >ACL >medial femoral condyle; in one patient the measure showed telomere length of medial femoral condyle >ACL > lateral femoral condyle.

Conclusions: From test 1, we found expression of asporin in ACL increased in the OA patients, it suggested degeneration of ACL in the OA patients. From test 2, we found that ACL injury would increase the varus angles of the knee. From test 3, we found that senescent degree in osteoarthritis knee is medial femoral condyle >ACL > lateral femoral condyle. We infered that ACL might degenerate or get injured at first, and then led to knee varus, and led to osteoarthritis at last.

Bone Biology

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FSTL3 MEDIATES EXERCISE-DRIVEN BONE FORMATION.

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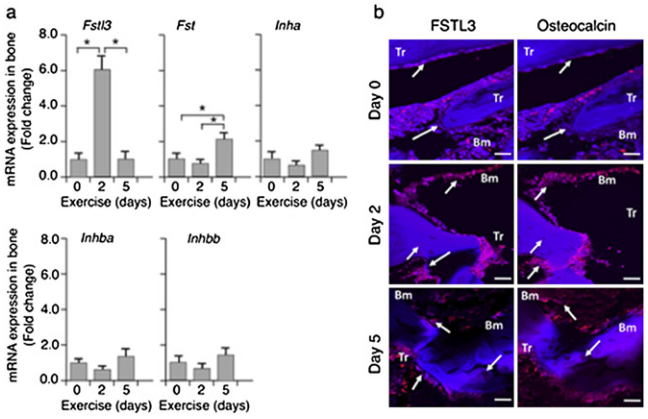

Objective: Exercise promotes bone remodeling through well-coordinated biochemical events. This is evident by the observations that bone forms in response to mechanical loading and resorbs following sustained unloading. Despite this well demonstrated phenomenon, how exercise drives the mechanoresponsiveness in bone is still elusive. Here, we provide evidences that Follistatin-like3 (FSTL3) could be a mechanoresponsive protein that that takes part in the exercise-driven bone formation.

Methods: Institutional Animal Care and Use Committee at OSU approved all experimental protocols. Female Sprague Dawley rats (12-14 wks, n=10), wild-type (WT, 10-12 wks, n=10) or homozygous FSTL3-/- C57Bl/6 mice (10-12 wks, n=10) were subjected to exercise by treadmill walking at 12 M/min (rats) or 8 M/min (mice) for 45 min/day. Following 0, 2, 5 or 15 days of exercise, animals were sacrificed and gene expression was analyzed by quantitative real time polymerase chain reaction (qrtPCR), Western blots, or immunohistochemistry of the trabecular bone/bone marrow at the distal ends of femurs. Bone mineral apposition rates of wild type or FSTL3-null mice were assessed using two fluorochromes, Calcein and Alizarin complexones injected on day 3 and 12, respectively, during the total exercise period of 15 days. On day 15, femurs of the mice were excised, acrylamide embedded, sectioned, and subjected to fluorescence microscopy to examine bone remodeling. Statistical analysis was performed by one-way ANOVA with Tukey's post hoc or T-test.

Results: Gene expression for Fstl3, Follistatin (Fst), Inhibin-Alpha (Inha), Inhibin-Beta-A (Inhba), and -Beta-B (Inhbb) by qrtPCR revealed that treadmill walking dramatically stimulated Fstl3 mRNA expression in the trabecular bone and bone marrow cells, reaching ~6 fold increase on day 2 ($p<0.05$) and declining by day 5, in rats and mice. However, the expression of Inha, Inhba, and Inhbb was unchanged (Fig 1A). In parallel, a robust increase in FSTL3 expression was observed in cells adjacent to trabecular bone, and in osteocalcin positive osteocytes located in cortical bone (Fig 1B). When Fstl3+/+ or Fstl3-/- mice were subjected to treadmill walking for 15 consecutive days, and injected with Calcein (day 3) and Alizarin (day 12) bone incorporation revealed limited bone deposition in both groups of non-exercised control mice. However, Fstl3+/+ mice when subjected to treadmill walking demonstrated a significant increase in bone deposition on the periosteal surface of the femur together with increase in the total mineral apposition rate (MAR; ~50% increase, $p < 0.05$). Similar treatment failed to induce exercise-induced bone formation and MAR increase in Fstl3-/- mice. Furthermore, FSTL3 null mice exhibited weaker femurs (ultimate strength FSTL3+/+ 3.2±0.4% vs. FSTL3-/- 2.4±0.3%, $p<0.003$) and brittle bones (fracture strain FSTL3+/+ 7.7±3.4% vs. FSTL3-/- 5±1.3%, $n = 7$, $p = 0.037$). Interestingly, circulating levels of FSTL3 increased in mice, humans and rats following 2 days (5.2±1.3) and 5 days (6.4±1.1 fold) of exercise, indicating a requirement of FSTL3 for the adaptive responses of the bone to mechanical loading.

Conclusions: Overall, the data suggest that FSTL3 is the first molecule identified that might be critical for bone formation and strengthening in response to mechanical loading of bones. This is evident by observations that genomic deletion of FSTL3 abolishes load-dependent bone formation, weakening of bones, and failure of upregulation of genes associated with bone deposition. The identification of FSTL3 as a mechanoresponsive protein provides a new paradigm for investigating exercise-driven bone formation and its use as a target to develop therapeutic drugs for treating bone diseases.

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A SPECIFIC SUBTYPE OF OSTEOCLASTS SECRETE FACTORS INDUCING BONE FORMATION

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Purpose: Osteoclasts are known to be important for the coupling process between bone resorption and formation, at least partially by secretion of bone anabolic factor(s), a phenomenon which could be involved in the subchondral plate thickening in OA .

The aims of this study were to address whether osteoclasts are the only source of anabolic factors, and how the ability to resorb, as well as how different matrices affect the release of bone anabolic factors from mature resorbing osteoclasts.

Methods: Human monocytes were isolated from cord blood or peripheral blood and differentiated into mature osteoclasts by treatment with M-CSF and RANKL. Conditioned medium was collected from macrophages, pre-osteoclasts, and mature functional and non-resorbing osteopetrotic osteoclasts on either bone, plastic, decalcified bone or dentine. Osteoclasts on bone and plastic were treated with vehicle or diphyllin, E64 or GM6001. Conditioned medium (CM) and corresponding non-conditioned medium (non-CM) was collected and pooled. Osteoclasts numbers were measured by TRACP activity. Bone resorption was evaluated by CTX-I and calcium release. The osteoblastic cell line 2T3 was treated with 50% of CM or non-CM for 12 days. Bone formation was assessed by Alizarin Red extraction.

Results: Only CM from mature osteoclasts induced bone formation, while CM from precursors or mature macrophages failed to do so. Non-resorbing osteoclasts generated from osteopetrosis patients showed very little resorption, yet a blunted, but still significant ability to induce bone formation by osteoblasts. Diphyllin and E64 all potently reduced resorption, while GM6001 did not. Collected CM from all the conditions induced bone formation significantly compared to their corresponding non-CM, with an 800% induction by vehicle CM. However, CM from diphyllin, cathepsin K inhibitor and E64 treated osteoclasts decreased the level of bone formation compared to CM from vehicle treated osteoclasts by approximately 50%, while CM from GM6001 treated osteoclasts equaled vehicle CM. Osteoclasts on either dentine or decalcified bone showed strongly attenuated anabolic capacities.

Conclusion: We present evidence that osteoclasts, both dependent and independent of their resorptive activity, secrete activities stimulating osteoblastic bone formation. Further understanding of these processes could shed light on the complicated interactions occuring in the subchondral plate during osteoarthritis.